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Title: Load-specific inflammation mediating effects of resistance training in older persons.

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#### Abstract: BACKGROUND

Little is known about the effects of resistance training (RT) on circulating cytokines in older adults. Also, dose-response relationships remain unclear. This study investigated the impact of RT at different external loads on circulating inflammatory mediators in older community-dwelling individuals.

#### METHODS

Fifty-six community-dwelling older (68±5 years) volunteers were randomized to 12 weeks of supervised RT (3x/week) at either high-resistance (HIGH, 8 Males, 10 Females, 2x10-15 repetitions at 80%1RM), low-resistance (LOW, 9 Males, 10 Females, 1x80-100 repetitions at 20%1RM), or mixed low-resistance (LOW+, 9 Males, 10 Females, 1x60 repetitions at 20%1RM followed by 1x10-20 repetitions at 40%1RM). Serum was available from 51 out of 56 participants at baseline and after 12 weeks for determination of IL-6, IL-8, IL-10, IL-1beta, sTNFR1, GM-CSF and IL-1ra.

#### RESULTS

12 weeks of RT significantly increased sTNFR1 from 2.48±0.57ng/mL to 2.58±0.59ng/mL (overall time-effect p=0.033) and LogIL-8 from 0.38±0.18pg/mL to 0.53±0.32pg/mL (overall time-effect p=0.007). No time X group interaction (p=0.916) was observed. In males of the HIGH group there was an increase in LogIL-8 (from 0.45±0.16pg/mL to 0.68±0.19pg/mL; p=0.005) and IL-1ra (from 68.60±24.12pg/mL to 79.56±29.07pg/mL; p=0.007). No significant changes were found for the other markers.

#### CONCLUSIONS

Our results show that 12 weeks of supervised RT induced an overall significant increase of circulating IL-8 and sTNFR1, independently from the external load applied. We suggest that exercising until volitional fatigue is the main trigger for exercise-induced responses. However, training at HIGH external load also increased anti-inflammatory IL-1ra in male participants, which might be beneficial in combating low-grade inflammation.



Dear Sir,

Hereby we submit a manuscript entitled: “**Load-specific inflammation mediating effects of resistance training in older persons.**” as Original Research Paper for publication in the **Journal of the American Medical Directors Association**.

In 2005, we demonstrated that a regular resistance training session is sufficient to obtain a significant acute increase in circulating IL-6 in older adults (*Bautmans et al. Gerontology. 2005;51:253-265*). We (*Forti et al. Age 2014;36:9704*) have also shown that 12 weeks resistance exercise significantly reduced the basal levels of IL-6, thus reflecting lower chronic low-grade inflammatory profile (CLIP). Despite the benefits of resistance training at high external load on muscle mass and strength, many clinicians hesitate to prescribe resistance exercise with high external resistances in older persons. As an alternative, resistance training at lower external resistance is often offered to older persons. However, studies investigating the dose-response relationship of resistance training on CLIP remain scarce.

Previously, we showed that high- and low- resistance exercise performed until muscle failure can be similarly effective in increasing muscle mass and basic strength. High-resistance training leads to a higher increase in 1RM strength than low-resistance training, but this difference disappeared when using a mixed low-resistance (LOW+) protocol in which the resistance was intensified within a single exercise set (*Van Roie et al. Experimental Gerontology 2013; 48:1351-61*). In the present study, we compared the effects of 12 weeks of resistance training using three different external loads (HIGH, LOW+ or LOW) on *basal* circulating inflammatory mediators in elderly persons (older than 60 years). The key finding in this study was that resistance training induced an overall significant increase of circulating IL-8 and sTNFR1, independently from the external load applied.

Fifty-six community-dwelling older (68±5 years) volunteers were randomized to 12 weeks of resistance training (3x/week) at either high-resistance (HIGH, 2x10-15 repetitions at 80% 1RM), low-resistance (LOW, 9 Males, 10 Females, 1x80-100 repetitions at 20% 1RM), or mixed low-resistance (LOW+, 9 Males, 10 Females, 1x60 repetitions at 20% 1RM followed by 1x10-20 repetitions at 40% 1RM). Serum was available from 51 out of 56 participants at baseline and after 12 weeks (24h-48h after the last training) for determination of IL-6, IL-8, IL-10, IL-1beta, sTNFR1, GM-CSF and IL-1ra.

Our results show that 12 weeks of resistance training induced an overall significant increase of circulating IL-8 and sTNFR1, independently from the external load

applied. We suggest that exercising until volitional fatigue is the main trigger for exercise-induced immune responses. However, training at HIGH external load also increased anti-inflammatory IL-1ra in male participants, which might imply additional benefits in combating low-grade inflammation. These findings provide additional new insight into the underlying mechanisms of exercise-induced changes in circulating inflammatory mediators. Consequently, specific attention to exercise modalities might become a major attention point for designing exercise interventions in older adults. Therefore, we think that our manuscript describes important information for the readers of the Journal of the American Medical Directors Association.

This study is part of the research line concerning sarcopenia, inflammation and improvement of muscle performance of the Frailty in Ageing department ([www.vub.ac.be/FRIA](http://www.vub.ac.be/FRIA)) at the Vrije Universiteit Brussel (Brussels, Belgium).

All authors meet the criteria for authorship.

Yours sincerely,

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**Load-specific inflammation mediating effects of resistance training in older persons.**

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# Load-specific inflammation mediating effects of resistance training in older persons.

## ABSTRACT

## BACKGROUND

Little is known about the effects of resistance training (RT) on circulating cytokines in older adults. Also, dose-response relationships remain unclear. This study investigated the impact of RT at different external loads on circulating inflammatory mediators in older community-dwelling individuals.

## METHODS

Fifty-six community-dwelling older ( $68 \pm 5$  years) volunteers were randomized to 12 weeks of supervised RT (3x/week) at either high-resistance (HIGH, 8 Males, 10 Females, 2x10-15 repetitions at 80% 1RM), low-resistance (LOW, 9 Males, 10 Females, 1x80-100 repetitions at 20% 1RM), or mixed low-resistance (LOW+, 9 Males, 10 Females, 1x60 repetitions at 20% 1RM followed by 1x10-20 repetitions at 40% 1RM). Serum was available from 51 out of 56 participants at baseline and after 12 weeks for determination of IL-6, IL-8, IL-10, IL-1 $\beta$ , sTNFR1, GM-CSF and IL-1ra.

## RESULTS

12 weeks of RT significantly increased sTNFR1 from  $2.48 \pm 0.57$  ng/mL to  $2.58 \pm 0.59$  ng/mL (overall time-effect  $p=0.033$ ) and LogIL-8 from  $0.38 \pm 0.18$  pg/mL to  $0.53 \pm 0.32$  pg/mL (overall time-effect  $p=0.007$ ). No time X group interaction ( $p=0.916$ ) was observed. In males of the HIGH group there was an increase in LogIL-8 (from  $0.45 \pm 0.16$  pg/mL to  $0.68 \pm 0.19$  pg/mL;  $p=0.005$ ) and IL-1ra (from  $68.60 \pm 24.12$  pg/mL to  $79.56 \pm 29.07$  pg/mL;  $p=0.007$ ). No significant changes were found for the other markers.

## CONCLUSIONS

Our results show that 12 weeks of supervised RT induced an overall significant increase of circulating IL-8 and sTNFR1, independently from the external load applied. We suggest that exercising until volitional fatigue is the main trigger for exercise-induced responses. However, training at HIGH external load also increased anti-inflammatory IL-1ra in male participants, which might be beneficial in combating low-grade inflammation.

## KEYWORDS

**Cytokines; Aged; Resistance training; Training load**

## 1 INTRODUCTION

2 Regular physical exercise is suggested to be beneficial to the immune system as an efficient  
3 tool in fighting off chronic low-grade inflammatory profile (CLIP)<sup>1</sup>. The beneficial effects of  
4 physical exercise on CLIP and age-related diseases are reported to be mediated by molecules  
5 produced by contracting skeletal muscles termed myokines<sup>2</sup>. In fact, exercise provokes an  
6 acute liberation of inflammatory cytokines, especially IL-6<sup>3</sup>, which is different from the acute  
7 phase response in pathologic conditions. The exercise-induced acute elevation in IL-6 is not  
8 preceded by increased TNF- $\alpha$  levels<sup>4</sup>, but is immediately followed by elevations in IL-1ra  
9 and sTNFR<sup>4</sup> (inhibiting IL-1 $\beta$  and TNF- $\alpha$ ), and the anti-inflammatory cytokine IL-10. Here,  
10 IL-6 is believed to have inflammation-reducing effects, by stimulating immune cells to  
11 produce anti-inflammatory cytokines<sup>5,6</sup>. On the long term, these acute exercise-induced  
12 increases in IL-6 at each exercise session and its accompanying release of inflammation-  
13 reducing cytokines are believed to reduce CLIP.

14  
15 The exercise-induced myokine production seems to be maintained at higher age and there is a  
16 general consensus among exercise immunologists that intensive aerobic exercise training is  
17 effective in inducing a robust cytokine response<sup>7,8</sup>. However, given the significant ageing-  
18 related loss of muscle mass and muscle strength, resistance training at high external load (i.e.  
19 2-3 sets of 8-12 reps at 70-85% of the one repetition maximum [1RM]) is of major  
20 importance at higher age<sup>9</sup>. Studies on the impact of resistance training on cytokine response in  
21 older persons are scarce compared to the extensive literature on aerobic exercise training. In  
22 2005, we demonstrated that a regular resistance training session is sufficient to obtain a  
23 significant acute increase in circulating IL-6 in older adults<sup>10</sup>. We<sup>11</sup> have also shown that 12  
24 weeks of resistance exercise significantly reduced the basal levels of IL-6, thus reflecting  
25 lower CLIP. This is consistent with the findings of Peake et al. who found a significant  
26 decrease in basal IL-6 levels following 12 months resistance training compared to control<sup>12</sup>.  
27 In contrast, Bruunsgaard et al.<sup>13</sup> reported no change in basal levels of IL-6, TNF- $\alpha$ , and

sTNFR1 in frail nursing home residents following 12 weeks of resistance training. This might have been due to the frail state of the participants which could have had a negative impact on the exercise-induced myokine production. Kapasi et al. also found no immune-enhancing effects of a 32-week combined exercise training in frail older subjects<sup>14</sup>.

Despite the benefits of resistance training at high external load on muscle mass and strength, many clinicians hesitate to prescribe resistance exercise with high external load in older persons<sup>15</sup>. As an alternative, resistance training at lower external load is often offered to older persons. Onambele-Pearson et al. reported a significant decrease in TNF- $\alpha$  levels with a low external (2-4 sets of 8-11 reps at 40% of 1RM) compared with a high-external load (2-4 sets of 8-11 reps at 80% of 1RM) protocol<sup>16</sup>, but studies investigating the dose-response relationship of resistance training on CLIP remain scarce. To the best of our knowledge, most studies using low-to-moderate-resistance exercise protocols only reduce the external load without substantially increasing the number of repetitions<sup>16-19</sup>. Taking into account the importance of training to volitional fatigue for optimizing muscular adaptations<sup>20</sup>, a training protocol with both a reduction in the external load as well as a substantial increase of the number of repetitions (until volitional fatigue) might be optimal. Previously, we<sup>21</sup> reported that 12 weeks of high resistance training (HIGH, 2 x 10-15 repetitions at 80% of 1RM) led to a higher increase in 1RM than low resistance training (LOW, 1x 80-100 repetitions at 20% of 1RM) in community-dwelling adults aged 60 and older. However, this difference disappeared when a mixed low-resistance (LOW+, 1 x 60 repetitions at 20% of 1RM, followed by 1 x 10–20 repetitions at 40% of 1RM) protocol was compared to HIGH. In addition, the HIGH, LOW and LOW+ resistance exercise programs had a similar outcome on muscle hypertrophy.

In the present study we compared the effects of 12 weeks of supervised resistance training at these three different external loads (HIGH, LOW and LOW+) on basal levels of inflammatory mediators in older adults. Therefore, the main objective of this study was to investigate the



impact of supervised resistance training at different external loads on peripheral serum circulatory inflammatory mediators in older community-dwelling individuals.

## **PARTICIPANTS AND METHODS**

### **Study Design and Participants**

This was a randomized intervention study. The recruitment strategy and main study procedures have been reported in detail previously<sup>21</sup>. Participants were excluded if they were involved in any structured endurance exercise and / or participated in resistance exercise during the last 6 months prior to the study, were suffering from hip or knee problems, showed unstable cardiovascular disease, neuromuscular disease or acute hernia. Briefly, 56 elderly volunteers were enrolled, and allocated to one of three training protocols: traditional high-resistance training (HIGH, n=18), low-resistance training (LOW, n=19), and mixed low-resistance training (LOW+, n=19) (see Figure 1). Randomization was stratified for sex, age, and baseline isometric knee extension strength. Five participants were excluded (one from the HIGH, three from the LOW and one from the LOW+ intervention group) from all statistical analyses since they lacked serum samples for cytokine analysis at either baseline or at 12 weeks (see Figure 1). Each participant gave a written informed consent after reading and understanding the risks and benefits associated with the study. The study protocol was approved by the local ethics committee in accordance with the Declaration of Helsinki.

### **Resistance Training Protocol**

The resistance training program took place at a local fitness and health centre for a duration of 12 weeks. The training program has been previously reported<sup>21</sup>. Briefly, after an initial familiarization session in which training techniques were explained and demonstrated, participants exercised 3 times weekly on nonconsecutive days for 12 weeks (total of 36 sessions). The exercises (leg press, leg extension and seated row) were performed on Technogym™ (Technogym, Gambettola, Italy) devices, designed for resistance training. Each

exercise session started with a brief warm-up (10 minutes) on a cycle ergometer (Technogym Bike Excite, Gambettola, Italy) or on a treadmill (Technogym Run Excite, Gambettola, Italy). Exercises were performed at a moderate speed with rest periods of 2 minutes in between and the training sessions were closely monitored by qualified fitness instructors. All participants were verbally encouraged to continue the exercises until failure (i.e. inability to perform more repetitions due to local muscle fatigue). Immediately after each individual exercise, participants graded their level of perceived exertion on the OMNI-Resistance Exercise Scale of Perceived Exertion (scale from 0 to 10)<sup>22</sup>. As described previously<sup>21</sup>, the 1RM was evaluated every 4 weeks (at baseline, before the first training session in week 5 and week 9, and after 12 weeks of training) and training loads were adapted accordingly. The training volume on the test sessions in week 5 and week 9 was reduced to only the leg extension exercise.

The exercise protocols were designed to be approximately equal in volume (% 1RM x number of repetitions). The HIGH resistance protocol (2 sets separated by 1 min intervals of 10-15 repetitions at 80% of 1RM) was based on ACSM's guidelines for resistance training<sup>23</sup>. These guidelines recommend performing at least one set to the point of failure for healthy individuals. In HIGH, the external resistance was initially set at 80% of 1RM. To ensure that maximal effort would be reached at the end of each set, participants were instructed to perform at least 10 to 15 repetitions. Two sets were performed with one minute of rest between sets. In the LOW resistance group, participants were instructed to complete 1 set of 80-100 repetitions at 20% of 1RM. Participants in the LOW+ group were instructed to complete first 60 repetitions at 20% of 1RM, and immediately afterwards (no rest), the external load was increased to 40% of 1RM and participants were instructed to perform 10-20 additional repetitions. During every exercise session, participants were asked to perform a maximum number of repetitions. If they could perform more repetitions than the target range (i.e. 80-100 or 10-15), the external load was increased. As described previously<sup>21</sup>, adherence (number of training sessions attended as a percentage of the total number of training sessions)

to the program was 95.7% in HIGH, 95.8% in LOW and 95.3% in LOW+, with no significant differences between groups.

## **Cytokine Assay**

Before the start (baseline) and at the end (at least 24h and maximum 48h after the last training session) of the 12 weeks resistance-training program serum samples were collected from all participants and stored at -80°C until assayed (simultaneously for all time points) for cytokines levels. Circulating serum levels of IL-8 (ultra-sensitive), IL-1 $\beta$ , GM-CSF, IL-1RA, IL-10 (ultra-sensitive) and sTNFR1, were measured separately using commercially available solid phase enzyme-linked immunosorbent assay kits (Lifetech. USA). Serum levels of IL-6 were performed using an IL-6 ultrasensitive singleplex Bead kit (Lifetech. USA). All reagents and calculations were applied according to the manufacturer's instructions. The sensitivities and the intra-assay coefficients of variation of the various cytokines, as well as the number of samples outside the detection limits are shown in Table 1. The baseline and 12 weeks samples from the same participants were run on the same plate to reduce variability.

## **STATISTICAL ANALYSIS**

The distribution of the data was checked using the Kolmogorov-Smirnov Goodness of Fit test, which revealed that sTNFR1 and IL-1ra followed a normal distribution while IL-6 and IL-8 were not normally distributed and were therefore log-transformed. As shown in table 1, most participants showed undetectable levels for IL-10, GM-CSF and IL-1 $\beta$ , and consequently no statistical analyses were performed for these cytokines. One-way ANOVA was used to test for baseline difference between the intervention groups. When a significant difference was detected, Bonferroni post-hoc tests were performed. Between groups differences in changes over time were analyzed with repeated measures ANOVA using time as within subjects' factor and group as between subjects' factor. To detect changes within groups (baseline vs. 12 weeks) paired sample t-test was employed. Statistical analysis was performed using IBM

SPSS version 22.0.0 software and Graphpad prism version 6.0. Significance was set *a priori* at two-sided  $p < 0.05$ . All data are presented as mean (standard deviation) except if otherwise indicated.

## RESULTS

The baseline characteristics of the 51 participants enrolled in this study are shown in Table 2. No significant differences among groups were detected at baseline for anthropometric parameters. On the other hand, there was a significant difference in LogIL-8 ( $p = 0.023$ ) and LogIL-6 ( $p = 0.023$ ) between the three intervention groups at baseline but not for the other cytokines. Post-hoc tests (see Table 2) revealed a significant difference between the HIGH and LOW+ group for LogIL-8 ( $p = 0.042$ ) and between the HIGH and LOW group for LogIL-6 ( $p = 0.019$ ). As previously reported, exercise induced a significant time by group interaction ( $p = 0.002$ ) in leg press 1RM, post hoc tests revealed a significantly higher increase in both HIGH (+46.2%  $\pm$  32.3%) and LOW+ (+39.2  $\pm$  20.7%) compared to the LOW (+23.1%  $\pm$  20.7%;  $p = 0.001$  and  $p = 0.006$ , respectively) <sup>21</sup>. Following supervised exercise, an overall significant increase in concentration of sTNFR1 (from 2.48 $\pm$ 0.57 ng/mL at baseline to 2.58 $\pm$ 0.59 ng/mL at 12 weeks; overall time-effect  $p = 0.033$ ), and LogIL-8 (from 0.38 $\pm$ 0.18 pg/mL at baseline to 0.53 $\pm$ 0.32 pg/mL at 12 weeks;  $p = 0.007$ ) was observed (data not shown). Exercise-induced changes in sTNFR1 and LogIL-8 were not significantly different between groups (time X group interaction: sTNFR1,  $p = 0.916$  and IL-8,  $p = 0.569$ ). No significant changes were found for Log IL-6 and IL-1ra (see Table 3).

To further evaluate the influence of sex, we stratified our participants according to sex. We found an overall significant increase in IL-8 and sTNFR1 (overall time-effect respectively  $p = 0.010$  and  $p = 0.009$ ) in the male participants only (data not shown). When groups were analyzed separately, we observed a significant increase in LogIL-8 (from 0.45 $\pm$ 0.16 pg/mL at baseline to 0.68 $\pm$ 0.19 pg/mL at 12 weeks;  $p = 0.005$ ) and IL-1ra (from 68.60 $\pm$ 24.12 pg/mL at

baseline to  $79.56 \pm 29.07$  pg/mL at 12 weeks;  $p = 0.007$ ) in males in the HIGH group (see Figure 2). In addition, a between group effect ( $p = 0.012$ ) was observed for LogIL-8 in the HIGH group when comparing male and female (see Figure 2). No significant within group effect was observed for females, nor for the other cytokines whether male or female.

## DISCUSSION

In this study we compared the effects of 12 weeks of supervised resistance training at three different external loads (HIGH, LOW and LOW+) on basal levels of inflammatory mediators in elderly persons. We found an overall significant increase in IL-8 and sTNFR1 following training, but no differences according to group allocation. When stratified for sex, we found a significant increase in IL-8 ( $p=0.010$ ) and sTNFR1 ( $p=0.009$ ) in the male participants only. Investigating within group effect following training we registered a significant increase in IL-8 and IL-1ra levels in males in the HIGH group. We observed no significant change in IL-6 levels after training. Our study corroborates previous findings by other investigators who reported no significant changes in basal levels of IL-6 following exercise<sup>16,18,24-26</sup>. In contrast, we recently demonstrated that 12 weeks of intensive strength training was sufficient to reduce basal IL-6 levels in untrained community-dwelling elderly individuals (older than 60 years)<sup>11</sup>. This is in line with other reports, that, using different training loads, also found a reduction or a trend towards reduced basal IL-6 levels after exercise<sup>10,12,19</sup>. It is important to note that fewer muscle groups were exercised (leg press, leg extension and seated row) in our current study compared to our previous study (leg press, leg abductor, leg adductor, vertical traction, chest press, and shoulder press)<sup>11</sup>. Moreover, the resistance training program in our previous study included 3 series of 10 repetitions at 70-80% of 1RM for each muscle group, compared to only 2 series of 10-15 repetitions at 80% of 1RM in our current study.

Interestingly, there was a significant increase of the anti-inflammatory cytokine IL-1ra following 12 weeks strength training in males in the HIGH intervention group, which might

imply additional benefits in combating low-grade inflammation. Since studies reporting on basal levels of IL-1ra following resistance training in the elderly are scarce<sup>7,27</sup>, our findings and the underlying mechanisms need to be confirmed in future studies.

TNF- $\alpha$  is modulated by soluble TNF- $\alpha$  receptors and these receptors are up-regulated on exposure to TNF- $\alpha$  and are shed into the circulation. The precise role of these soluble receptors (sTNFR1 and sTNFR2) is not yet completely understood. They can bind TNF- $\alpha$  in the circulation and may either attenuate its bioavailability or stabilize it thus prolonging its normal short half-life<sup>28</sup>. Soluble TNFR1 levels may actually reflect the overall nature of the inflammatory state. Despite this, the role of sTNFR1 in an exercise context is not known with certainty and needs to be distinguished from that related to infection. Following exercise, IL-6 has been shown to stimulate the production of well-known circulating anti-inflammatory cytokines (IL-1ra and IL-10) and cytokine inhibitors (soluble TNF- $\alpha$  receptors) but not IL-1 $\beta$  and TNF- $\alpha$ . Soluble TNFR is believed here to exert anti-inflammatory effects by inhibiting TNF- $\alpha$  signaling by competing for ligand-binding<sup>29</sup>. We observed an overall increase of basal levels of circulating sTNFR1 following exercise. In contrast, some groups have reported a significant decrease in sTNFR1 following exercise<sup>30-33</sup>. Interestingly, these reductions in sTNFR1 were not in isolation but were also reflected by significant decreases in other pro-inflammatory cytokines thus portraying a likely anti-inflammatory milieu.

Peripheral levels of IL-8 - a member of the CXC chemokine family- have been shown to increase in response to exhaustive exercise<sup>34</sup>. IL-8 is known to induce chemotactic effects via the chemokine receptor CXCR1 and angiogenic activity via CXCR2. Exercise has been demonstrated to provide an angiogenic stimulus within the working muscle and this has been reported to give rise to an increased capillarity<sup>35</sup>. The increased capillarity might be crucial in increasing blood flow to the muscles and in this way provide vital nutrients and oxygen. The ability of IL-8 to induce angiogenesis is different from its capacity to induce inflammation.

Increase in systemic IL-8 is seen following intensive exercise and it is thought that muscle derived IL-8 may be responsible for the exercise-induced angiogenesis<sup>34</sup>. Our study revealed an overall increase in circulating IL-8 following exercise. When stratifying according to sex, the increased IL-8 was significant only in male participants, which may explain the increase of IL-1ra in this group. The increased IL-8 level observed in our current study might be the result of an exercise-induced physiological adaptation other than a pro-inflammatory one, since the classical pro-inflammatory cytokine IL-1 $\beta$  remained undetectable after the training period. The role of exercise-induced changes in basal IL-8 levels needs further in-depth investigation.

The strength of this study is twofold; first, the study design is robust in nature (randomized study design), and second, compared with prior research on the impact of resistance training on basal circulating levels of cytokines, this study addresses a gap in the literature by systematically comparing resistance training at high (few repetitions) with low (many repetitions till volitional fatigue) external loads on circulating cytokines in the same cohort. However, this study is not without limitations. Firstly, there was no strict control group (a group that did not exercise), that might have masked some benefits resulting from the exercise intervention. Secondly, we did not control for or monitor dietary intake and energy consumption, which might have influenced cytokine responses to exercise<sup>36</sup>. Thirdly, in our study we were unable to report IL-10 data since most of the values were below the detection limit of the kit used (< 0.2 pg/mL). This result is in contrast with the report by Jankord and Jemiolo<sup>7</sup>, who showed that regular physical activity led to a significant increase in IL-10. However, it must be noted that the participants in the study by Jankord and Jemiolo<sup>7</sup> were healthy males corresponding to the SENIEUR criteria with a high level of physical activity (which is related to higher levels of circulating IL-10). Finally, although the detection limit of the ELISA kits used were <3 pg/mL for GM-CSF and <0.06 pg/mL for IL-1 $\beta$  respectively, most participants showed undetectable levels and consequently we were unable to perform

statistical analyses for these cytokines. However, Ostrowski et al. (Ostrowski *et al.* 1999) even though using an ELISA kit with a sensitivity of <0.1 pg/mL (compare with <0.06 pg/mL in our current study) reported no change in IL-1 $\beta$  following exercise.

## **CONCLUSION**

Our results show that 12 weeks of supervised resistance training induced an overall significant increase of circulating IL-8 and sTNFR1, independently from the external load applied. We suggest that exercising until volitional fatigue is the main trigger for exercise-induced immune responses. However, training at HIGH external load also increased anti-inflammatory IL-1ra in male participants, which might imply additional benefits in combating low-grade inflammation.

## **DECLARATION OF INTEREST**

We would like to acknowledge the Research Foundation Flanders, Belgium (FWO-Vlaanderen), as E. Van Roie was a Ph.D. Fellow of FWO. Also, the authors are very grateful to Sports and Health Center Respiro (Rotselaar, Belgium) for the use of the training equipment. The authors have no other declarations of interest to report.



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## LEGEND TO THE FIGURES

**FIGURE 1** - Flowchart of study participants

**FIGURE 2** – Change in Cytokines Concentrations Accordingly to Sex. \*Significant within group effect ( $p < 0.05$ ); \$significant between group effect ( $p < 0.05$ ).

Table 1

Table 1-Performance characteristics of ELISA kits

Cytokine	Sensitivity pg/ml	Intra-assay %CV			Inter-assay %CV			Undetectable at baseline (n)			Undetectable at 24 weeks (n)		
		L	N	H	L	N	H	HIGH	LOW	LOW+	HIGH	LOW	LOW+
IL-6	<0.05		7.59			9.99		0	0	0	0	0	0
IL-10	<0.2	4.5	2.8	2.6	7.8	7.5	7.5	11	7	9	11	10	7
IL-1β	<0.06	6.4	6.5	6.8	7.2	7.0	6.5	16	15	17	16	18	18
IL-8	<0.1	4.1	6.0	7.3	6.2	7.8	8.8	0	0	0	0	0	0
IL-1ra	4		4.8	4.1		7.1	9.1	0	0	0	0	0	0
sTNFR1	50	1.7		6.5	5.7		8.9	0	0	0	0	0	0
GM-CSF	<3	5.8	4.0	7.3	4.7	4.3	5.4	17	13	17	16	15	16

L= low; N = normal; H = high; CV = coefficient of variation.

Table 2- Overview of Participant characteristics at baseline

Variable	HIGH (n=17)	LOW (n=16)	LOW+ (n=18)	P
Sex (F/M)	9/8	9/7	9/9	
Age (years)	67.86±4.36	68.85±5.34	67.61±6.02	0.77 <sup>a</sup>
Weight (Kg)	71.66±11.67	75.56±15.50	77.41±11.96	0.43 <sup>a</sup>
Height (m)	1.67±0.08	1.64±0.10	1.67±0.08	0.64 <sup>a</sup>
BMI (Kg/m <sup>2</sup> )	25.57±2.90	27.79±4.28	27.72±3.10	0.12 <sup>a</sup>
1RM Leg Press (kg)	101.54±57.60	110.96±60.80	99.31±39.50	0.80 <sup>a</sup>
LogIL-8 (pg/mL)	0.48±0.17	0.34±0.14	0.33±0.20*	<b>0.02<sup>a</sup></b>
sTNFR1 10 <sup>3</sup> (pg/mL)	2.35±0.54	2.54±0.57	2.56±0.63	0.51 <sup>a</sup>
LogIL-6 (pg/mL)	0.33±0.38	0.05±0.15 <sup>\$</sup>	0.19±0.25	<b>0.02<sup>a</sup></b>
IL-1ra (pg/mL)	59.39±28.63	55.52±24.35	73.15±43.83	0.28 <sup>a</sup>

Except when indicated, data are mean ± SD; High = High resistance training; LOW = Low resistance training; LOW+ = Mixed low-resistance training; <sup>a</sup>Results of one-way analysis of variance between baseline group means; \*significantly different from the HIGH group; <sup>\$</sup>significantly different from the HIGH group.



**Table 3- Training induced changes in circulating inflammatory markers in the different intervention groups (HIGH, LOW, and LOW+)**

Variable	HIGH	LOW	LOW+	Time Effect	Time x Group Interaction
<b>Log IL-8 pg/mL</b>	n=16	n=16	n=16		
Baseline	0.48±0.17	0.34±0.14	0.33±0.20	<b>P=0.007</b>	P=0.427
12 weeks	0.54±0.27	0.49±0.33	0.56±0.38		
<b>sTNFR1 10<sup>3</sup> pg/mL</b>	n = 17	n = 16	n = 18		
Baseline	2.35±0.54	2.54±0.57	2.56±0.63	<b>P=0.033</b>	P=0.916
12 weeks	2.42±0.54	2.66±0.58	2.65±0.65		
<b>Log IL-6 pg/mL</b>	n = 16	n = 16	n = 16		
Baseline	0.33± 0.38	0.05±0.15	0.19±0.29	P=0.261	P=0.220
12 weeks	0.18±0.24	0.08±0.22	0.17±0.28		
<b>IL-1ra pg/mL</b>	n = 17	n = 16	n = 18		
Baseline	59.39±28.63	55.52±24.35	73.15±43.83	P=0.105	P=0.942
12 weeks	65.14±40.94	65.25±36.33	80.58±53.54		

Except if indicated, data are mean ± SD; HIGH = high resistance training; LOW = low resistance training; LOW+ = mixed low-resistance training; IL-8= Interleukin 8; sTNFR1= soluble tumor necrosis factor receptor 1; IL-6= Interleukin 6 ; IL-1ra= Interleukin 1 receptor antagonist

Figure 1

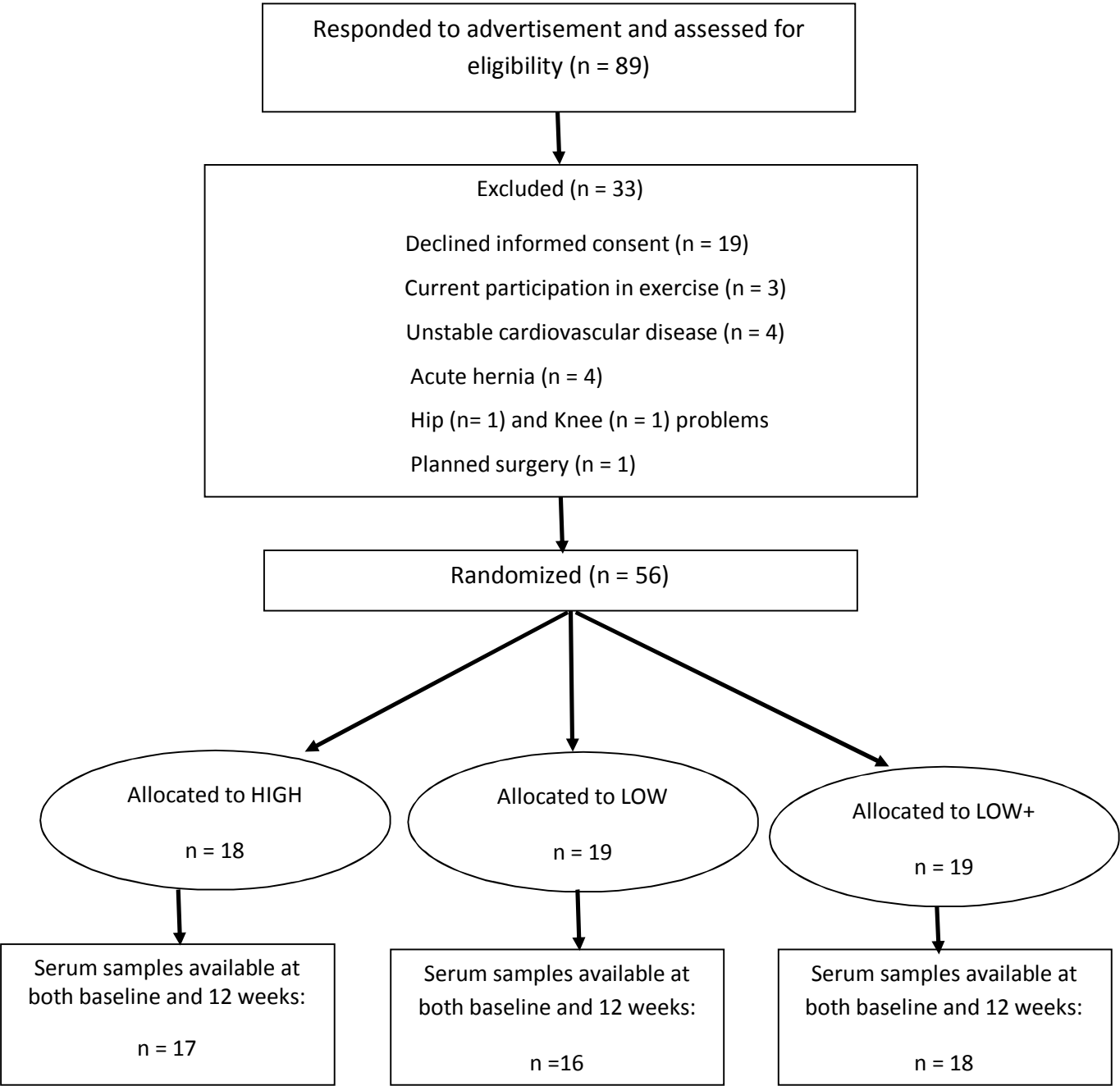


FIGURE 1 - Flowchart of study participants

Figure 2A

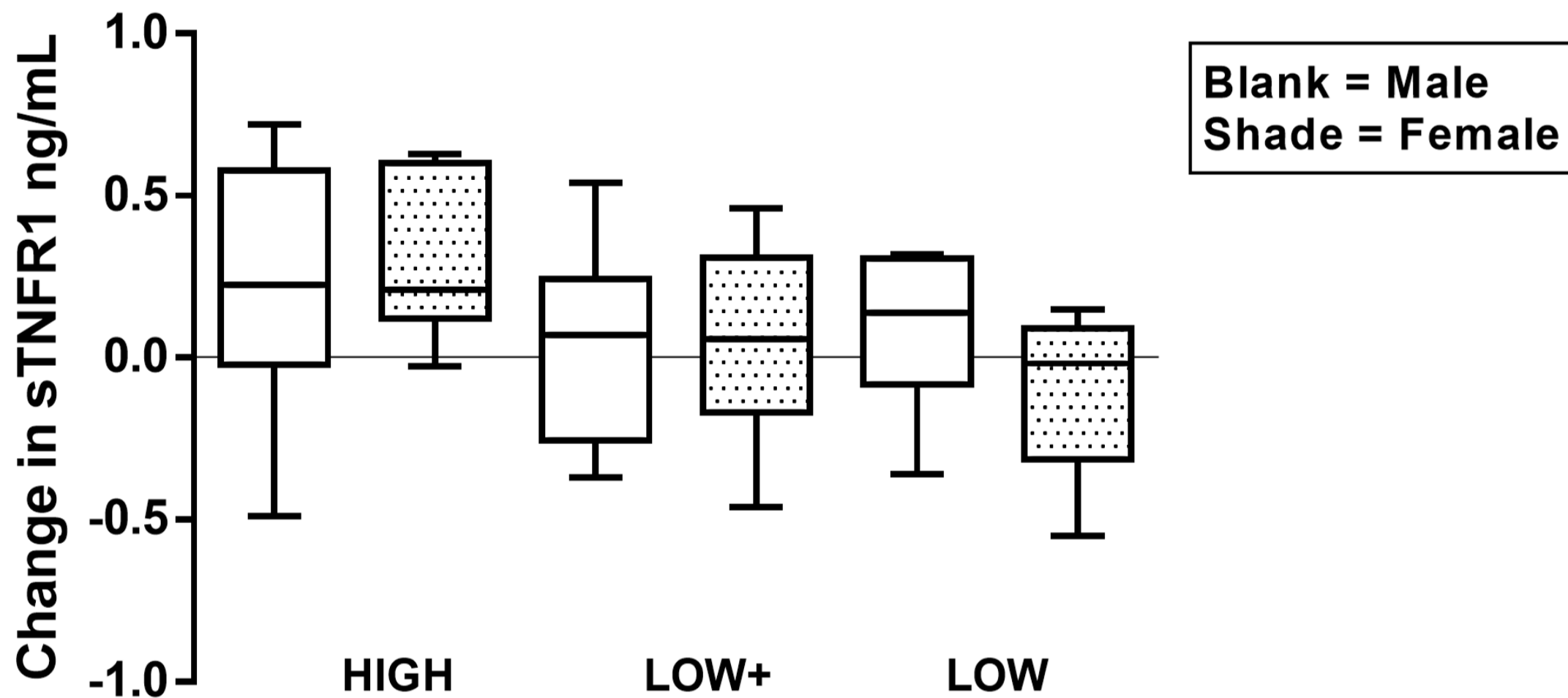


Figure 2B

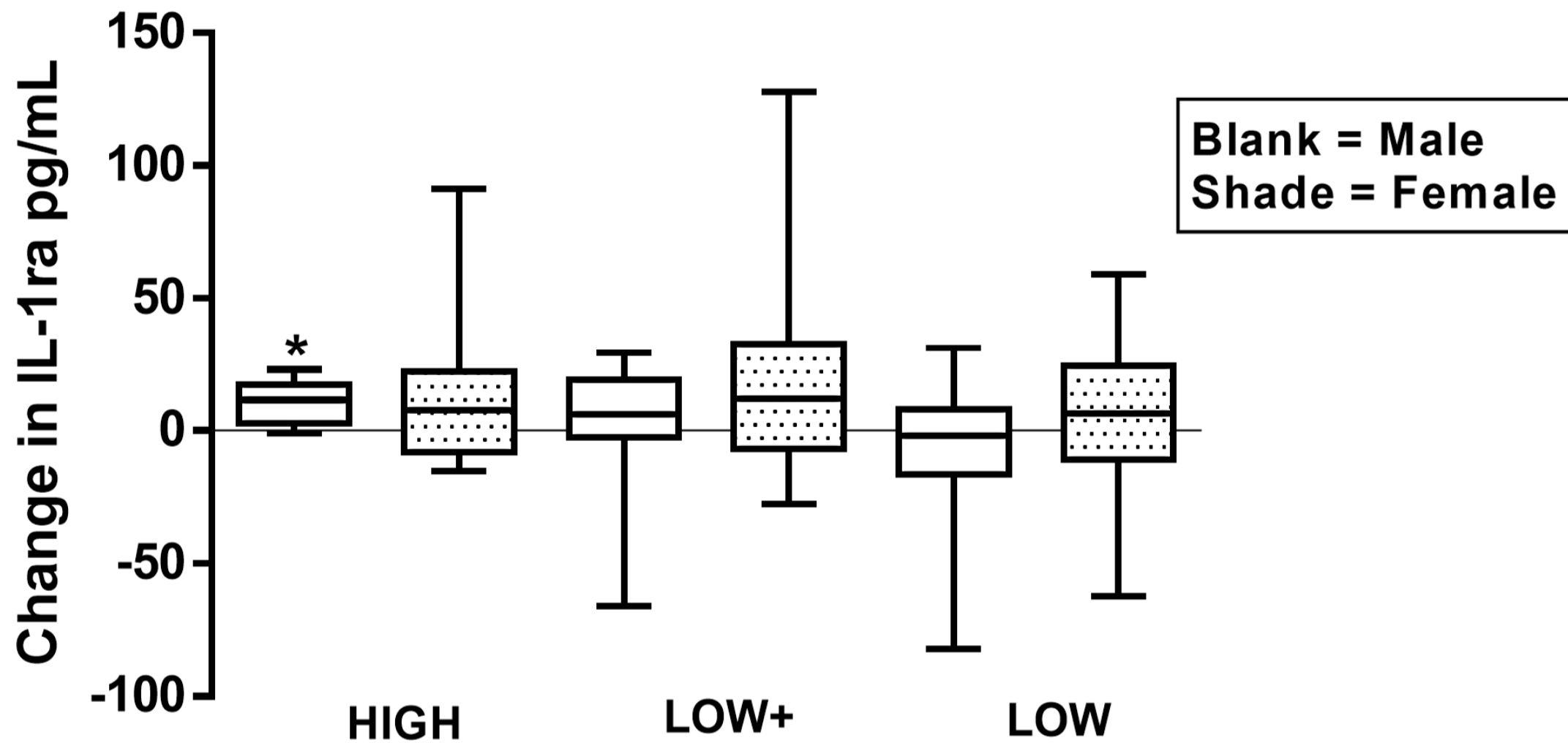


Figure 2C

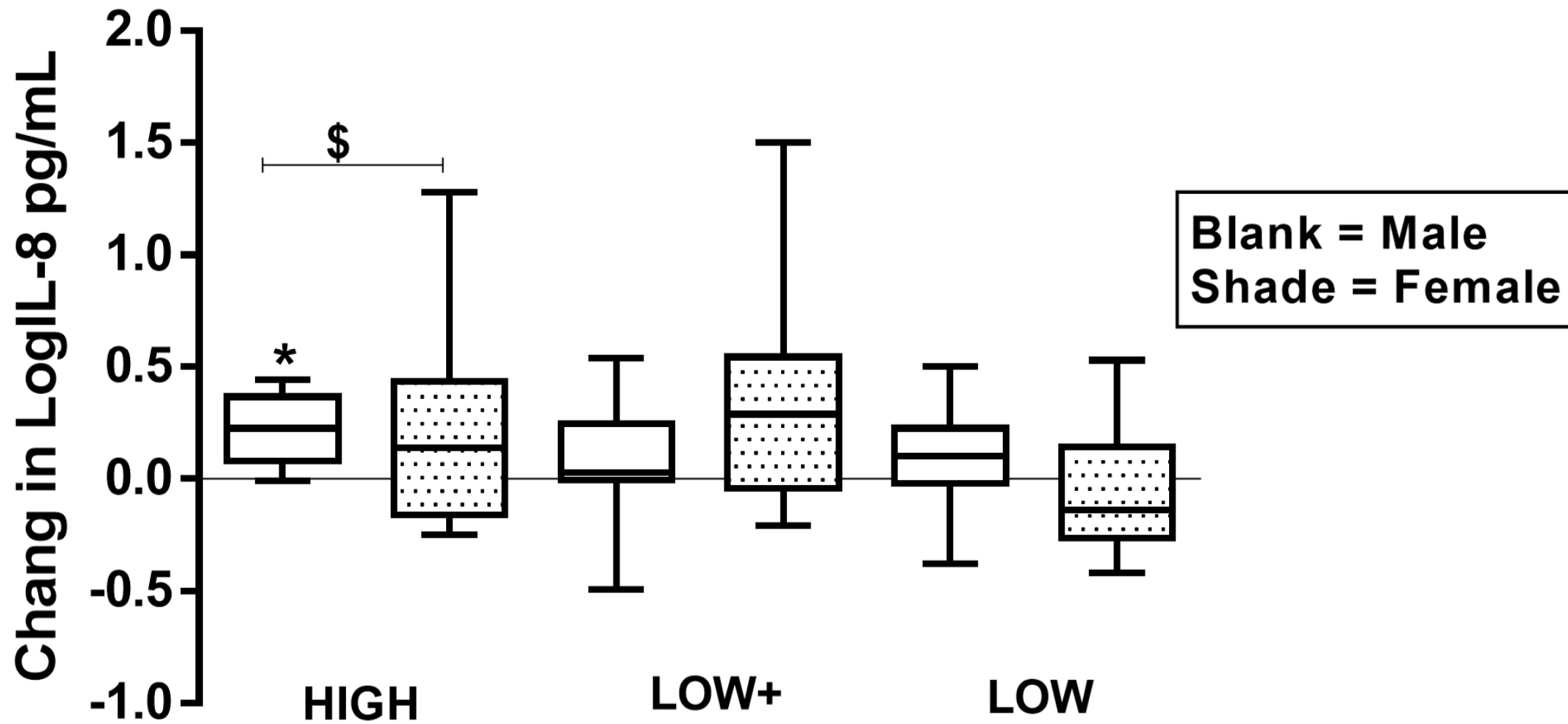


Figure 2D

